

Fig. 1

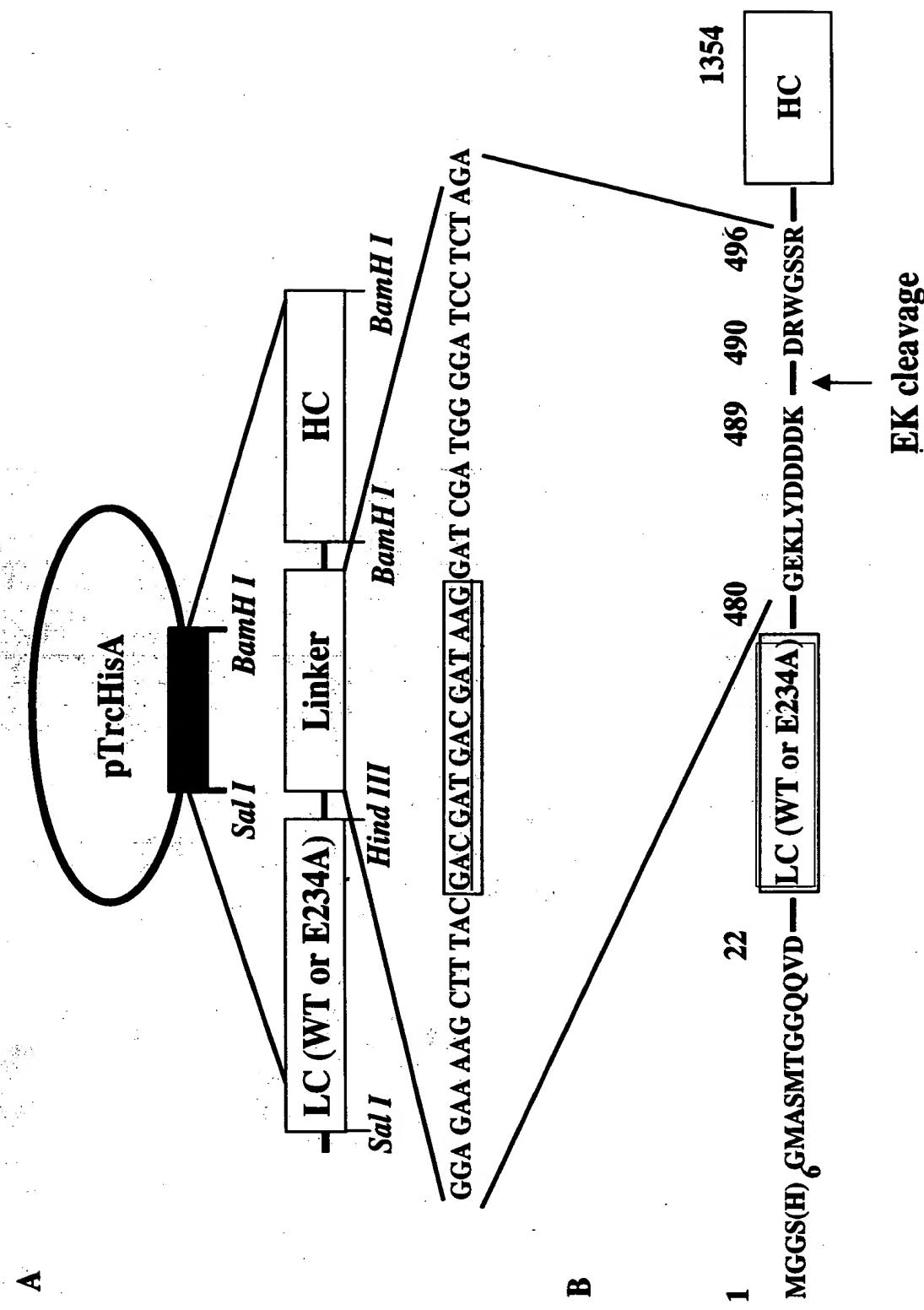


Fig. 2

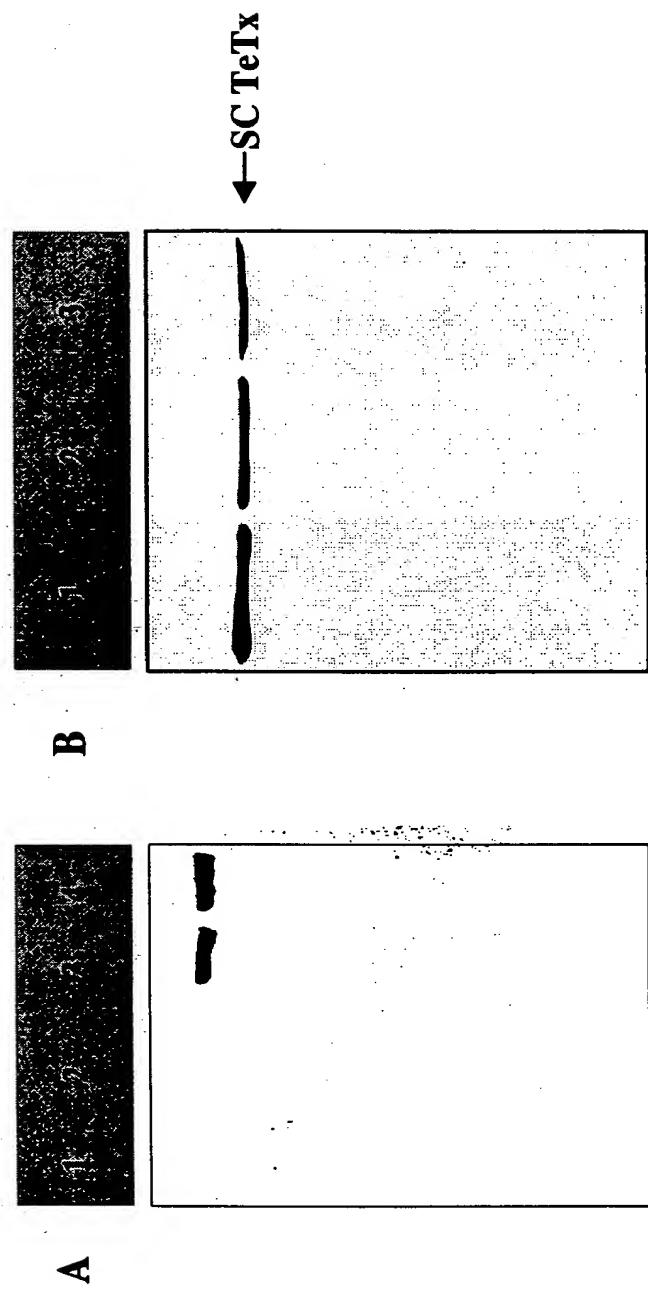


Fig. 3

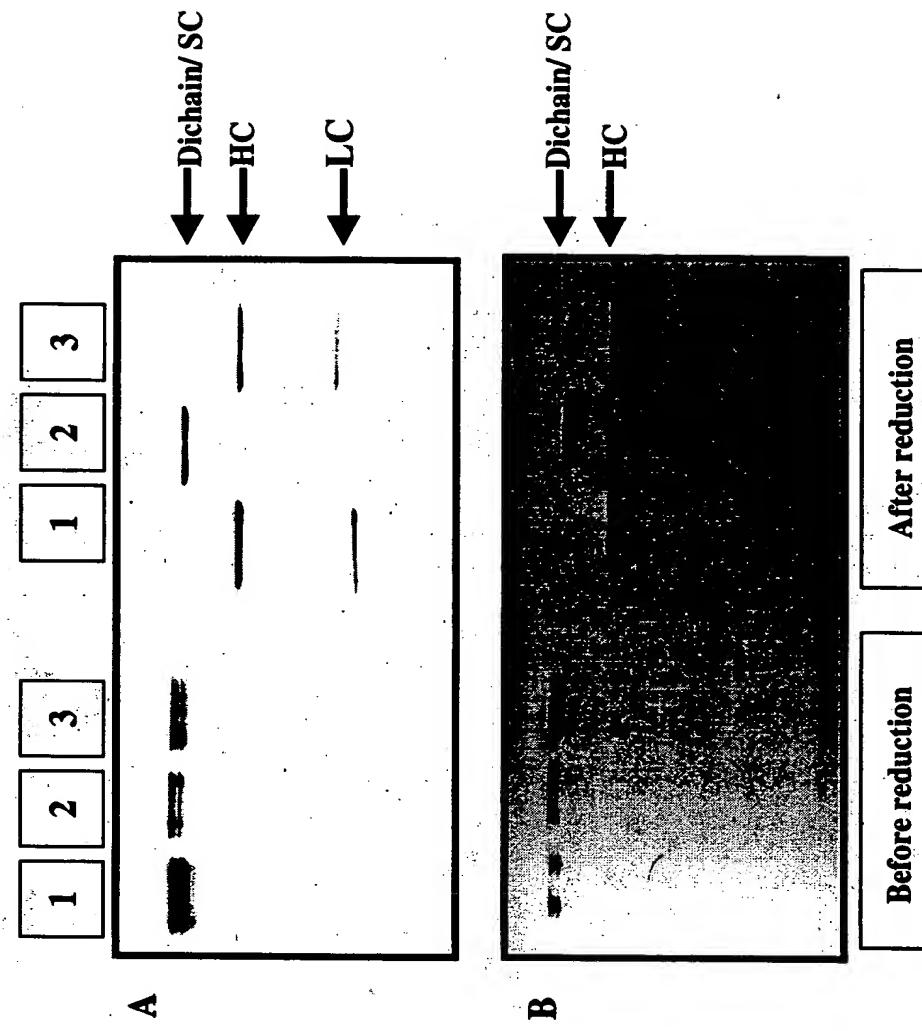
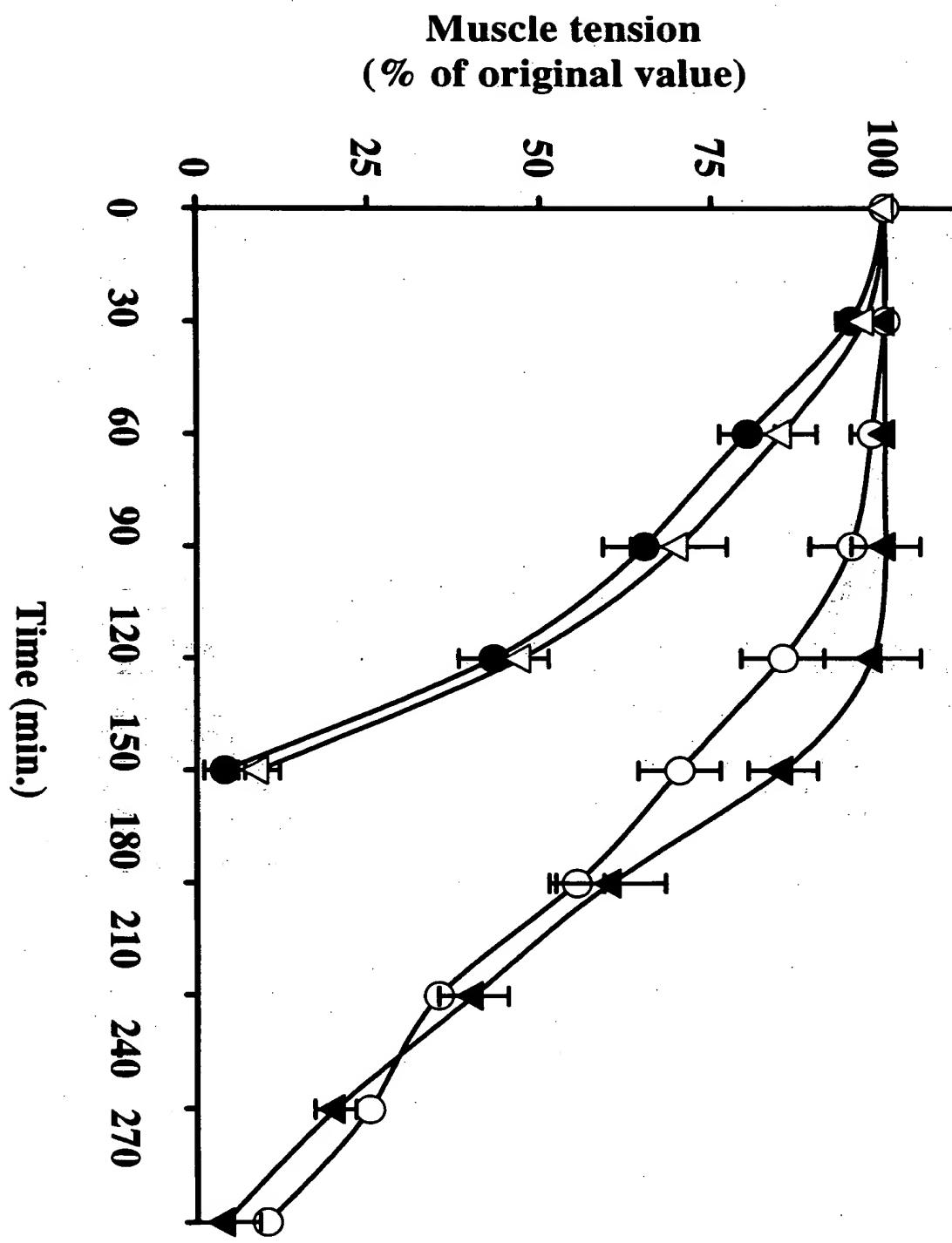


Fig. 4



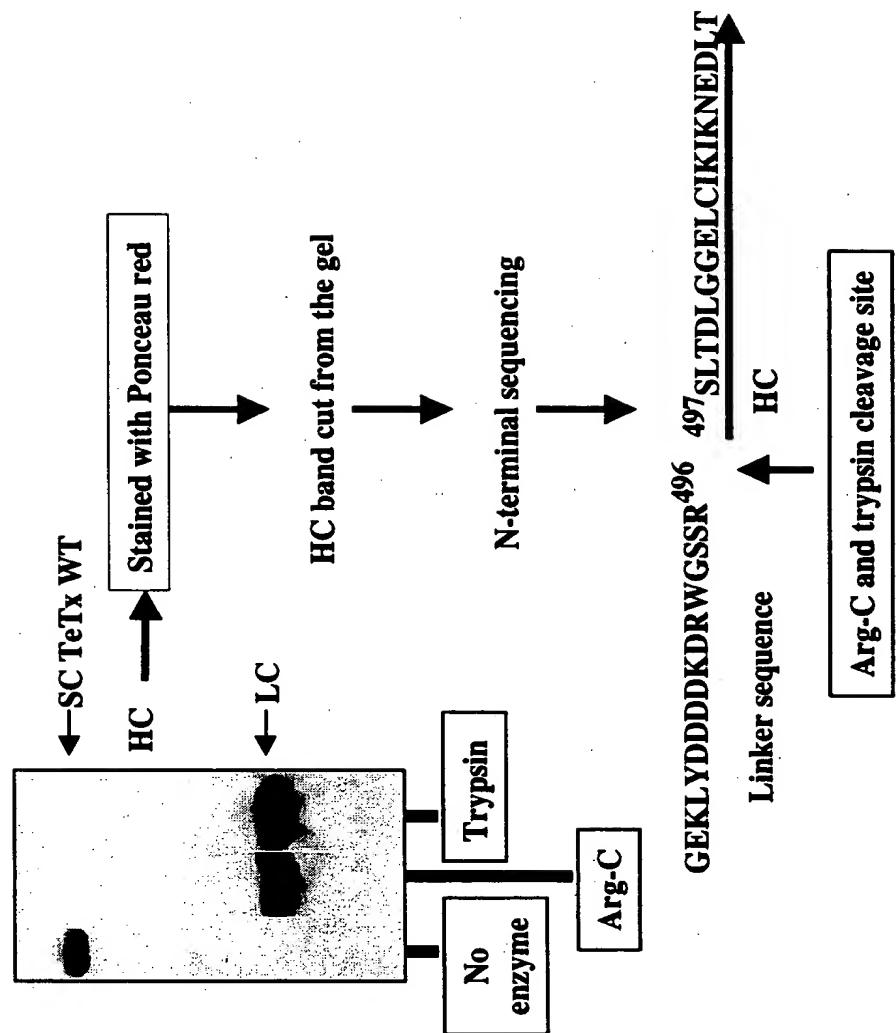


Fig. 5

Fig. 6

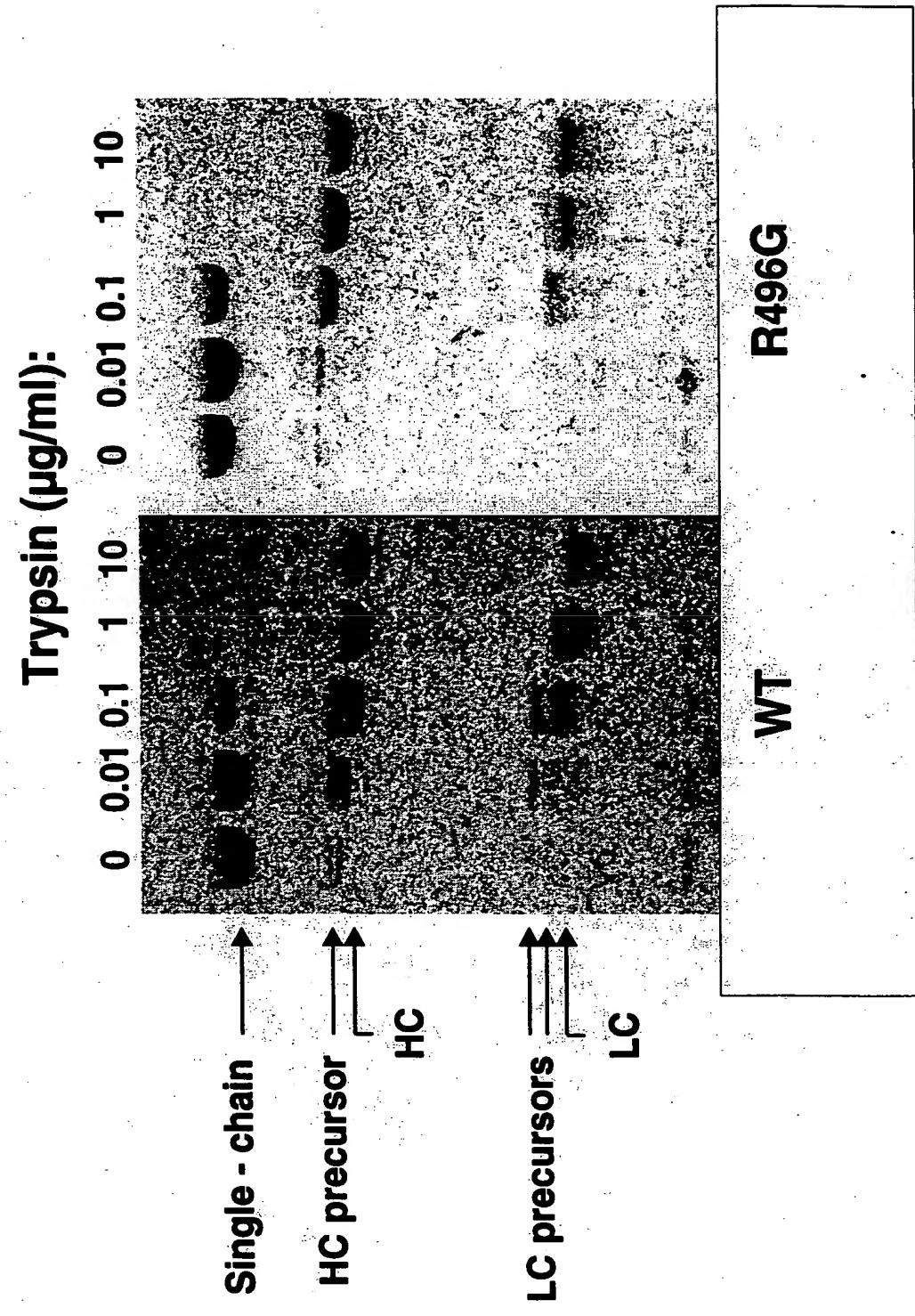


Fig. 7

Nicked mutant

Single-chain mutant

K⁺-evoked Ca²⁺-dep. release of
[¹⁴C] glutamate/5 min.
(% toxin-free control)

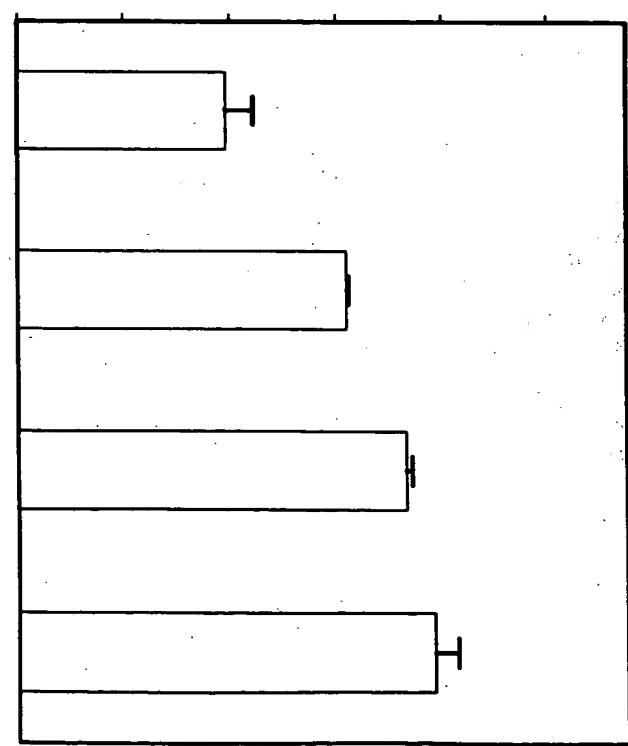
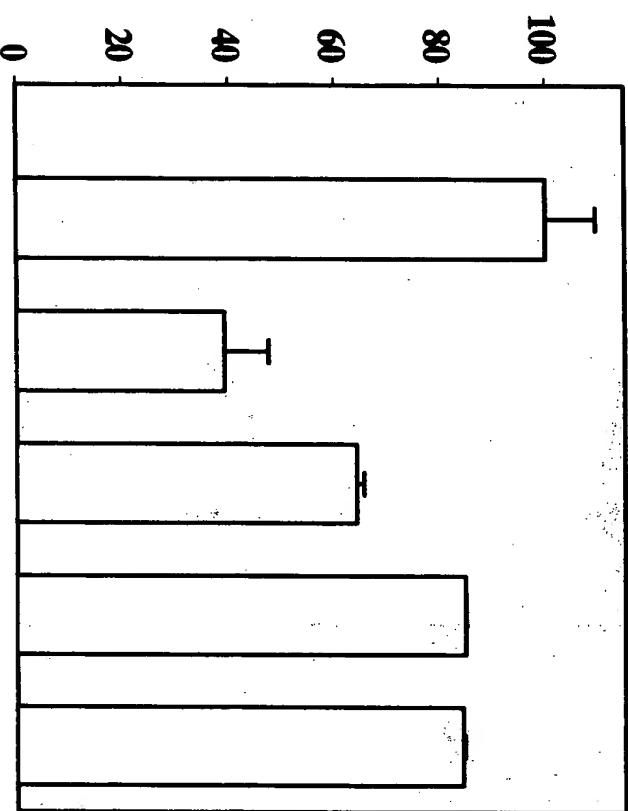


Fig.
8

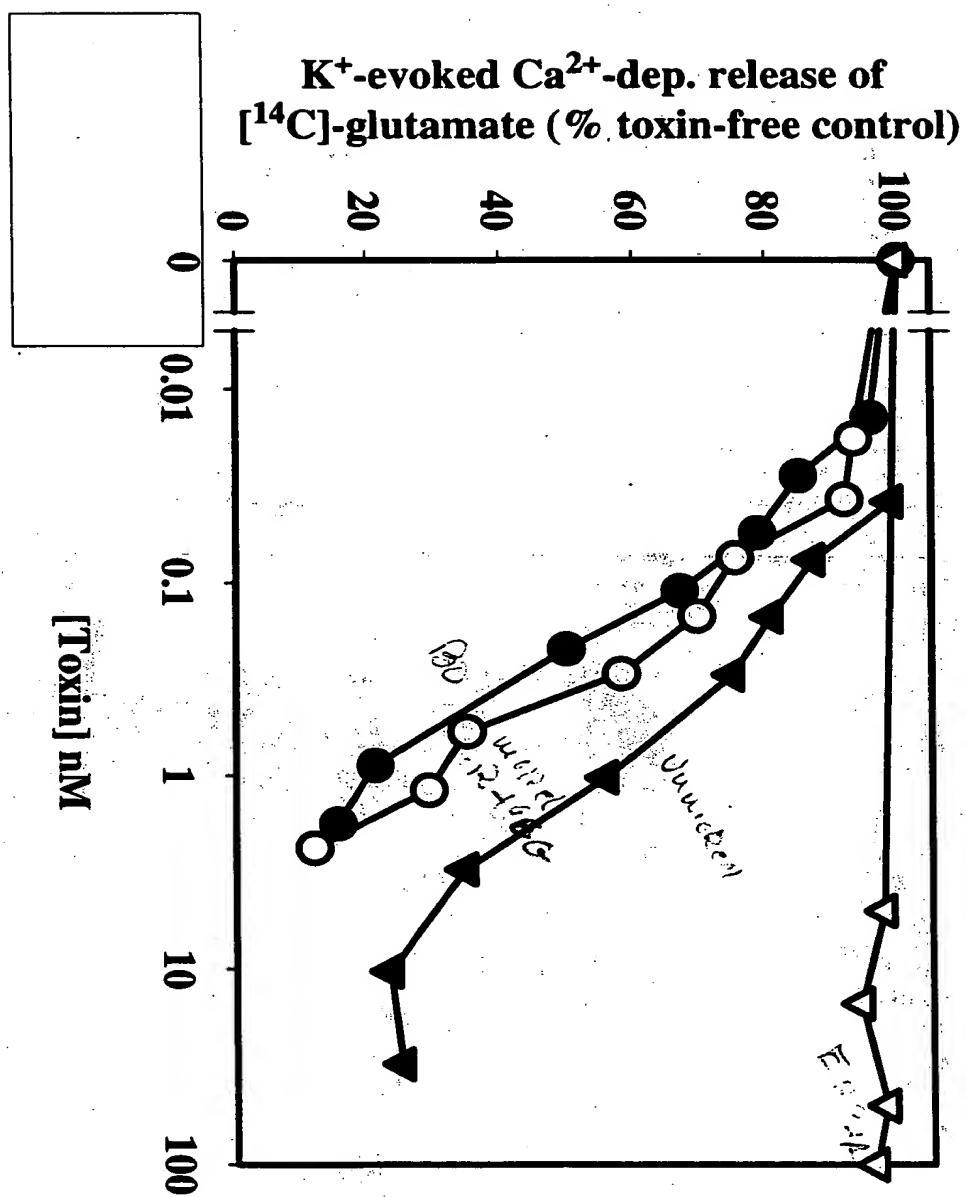
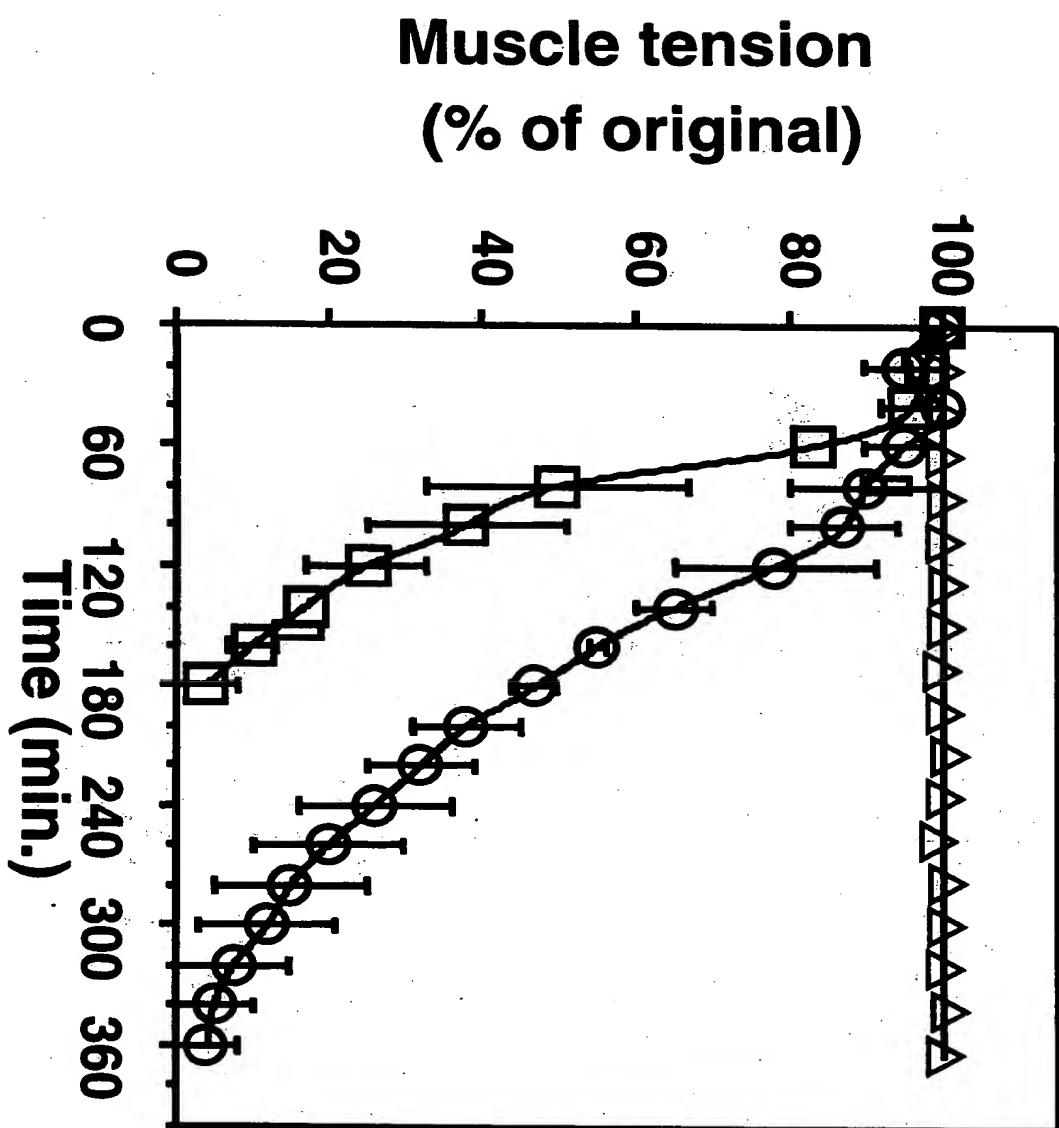


Fig. 9



0.05 4.5E-02 2.0E-02 5.0E-03

Fig. 10

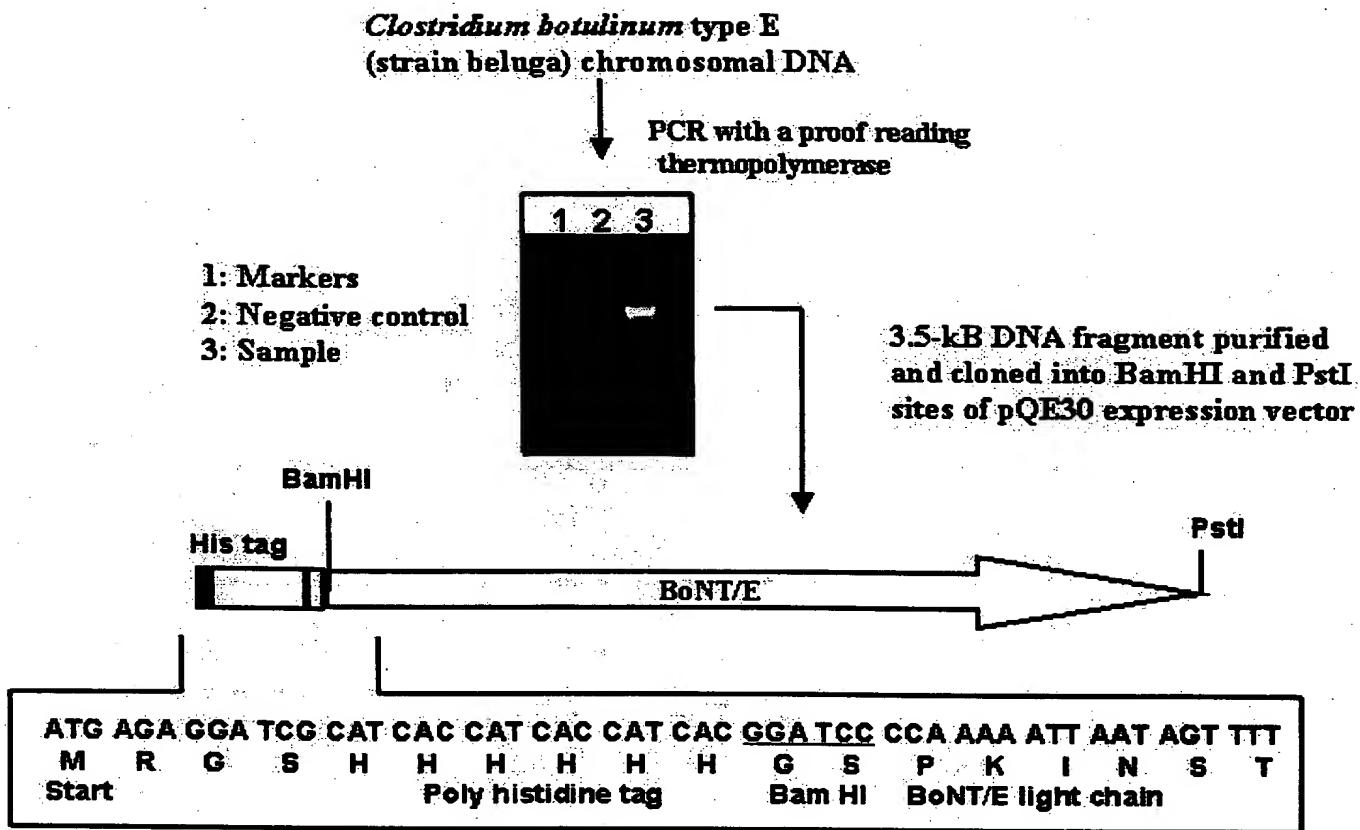


Fig. 11

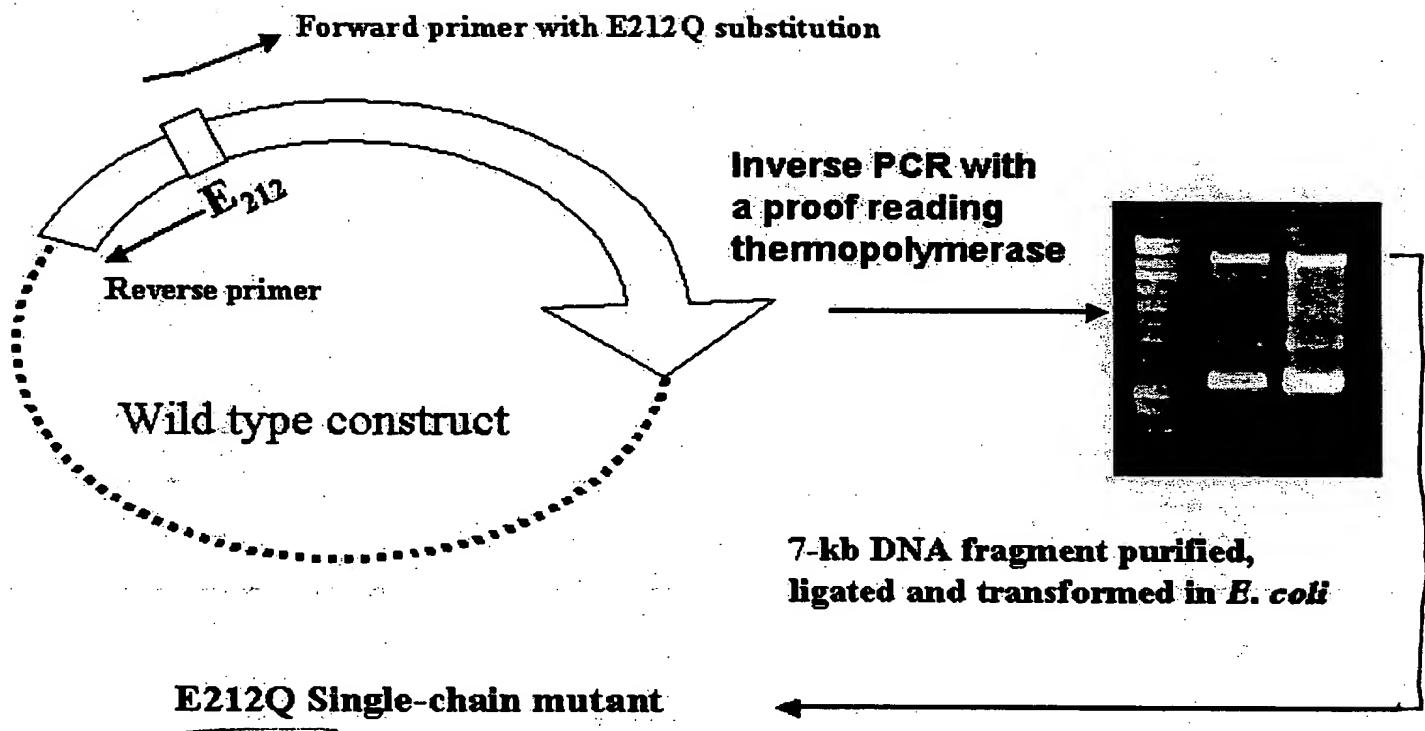


Fig. 12

Culture of *E. coli* M15[pQEESCwt]



Overnight induction with IPTG



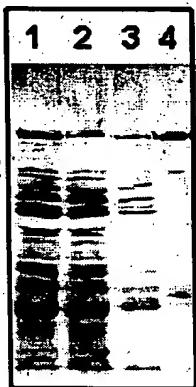
Harvest, extraction and centrifugation



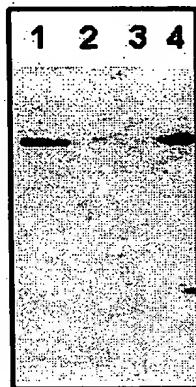
Metal chelate affinity chromatography

SDS-PAGE (reducing cond.)

- 1: Cell-free extract
- 2: Flow-through
- 3: Wash
- 4: Eluate
(0.15M imidazole)



Protein stained



Western blotted
with anti-His IgG

← 150 kDa
BoNT SC

Yield: 0.2 mg/L of culture

Fig. 13

- 1: BoNT/E from *Clostridium botulinum*
2: Un-nicked recomb. BoNT/E
3: Nicked recomb. BoNT/E
M: Markers

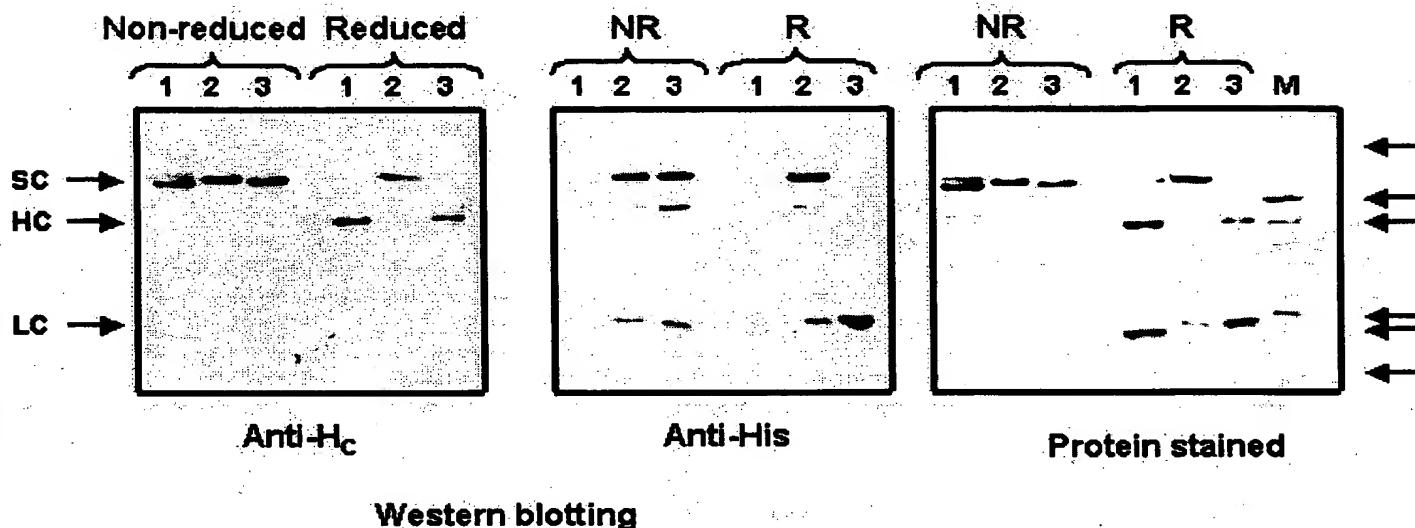


Fig. 14

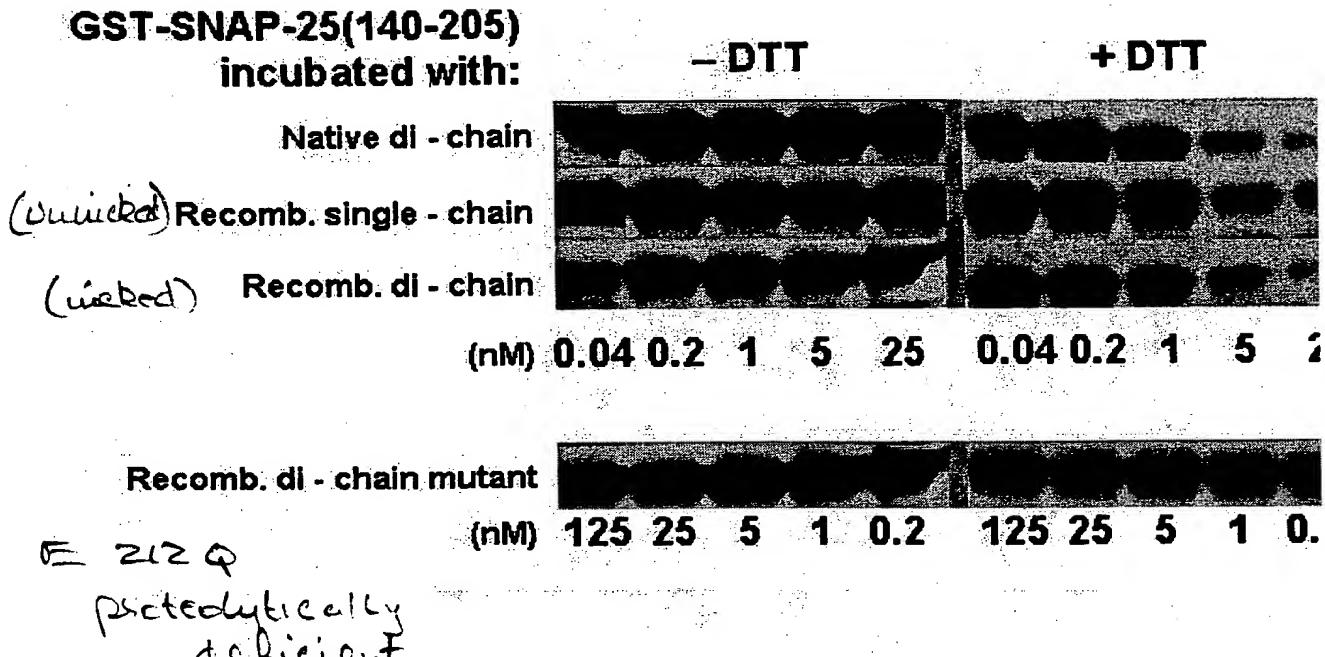
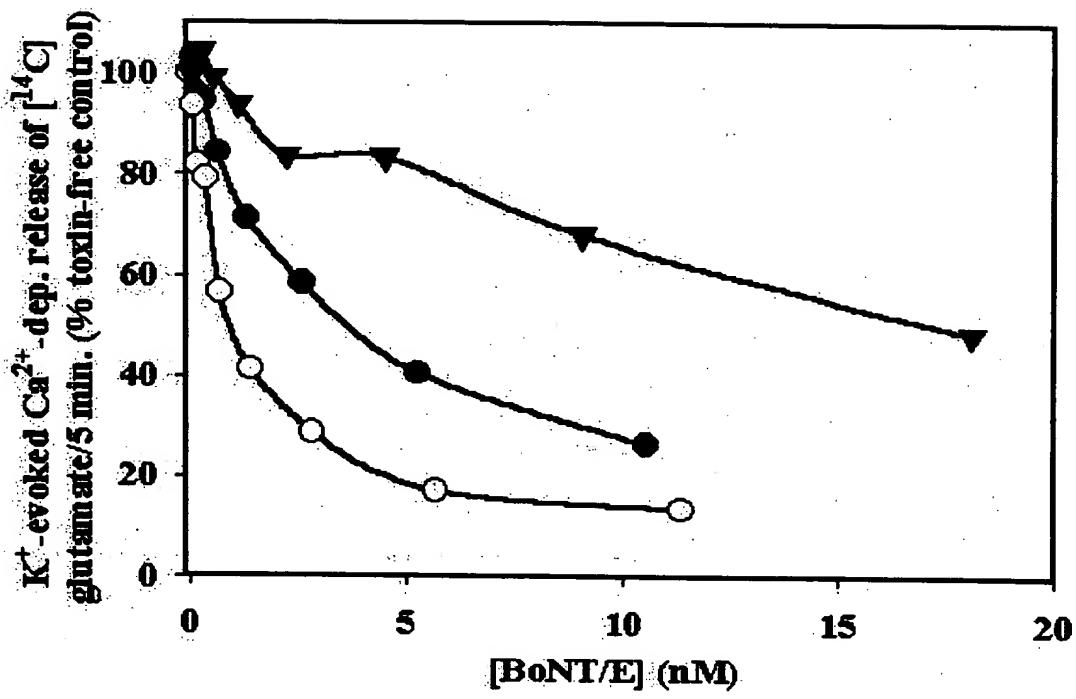


Fig. 15



• native
○ wicked seconds.
▼ unwicked seconds.

Cerebellar neurons.

Fig. 16

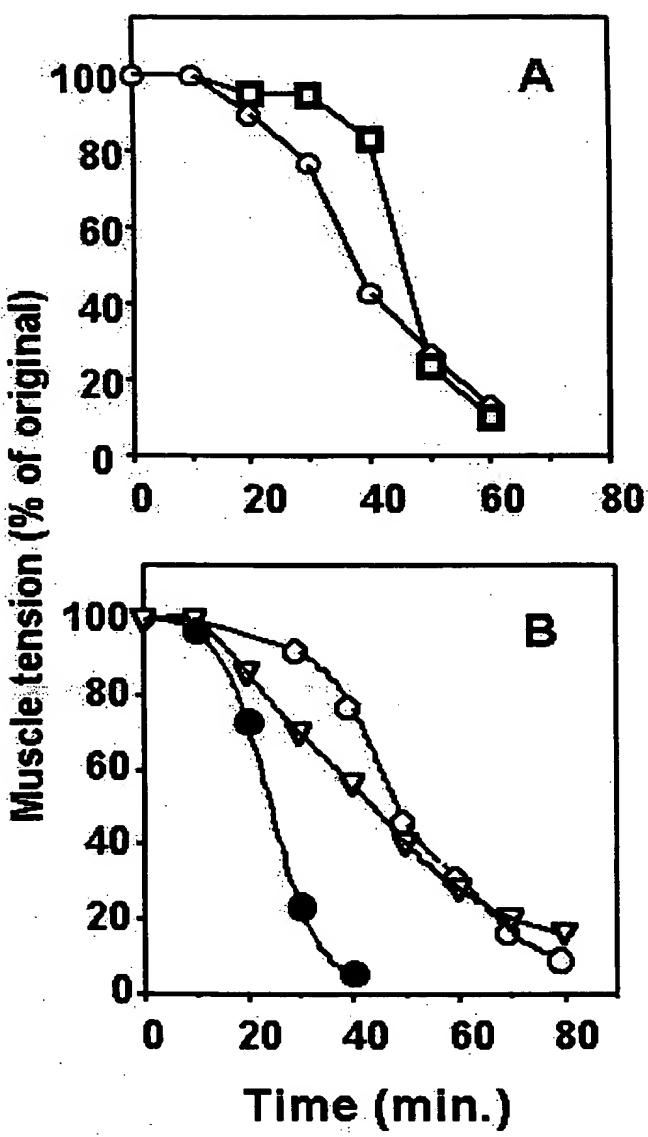


Fig. 17

